NEW SUBFAMILY CLASSIFICATION FOR THE RUTACEAE

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ABSTRACT

Thirty-four species representing all subfamilies and tribes of the Rutaceae Juss. were included in phylogenetic analyses that utilize six molecular data sets from five chloroplast markers, three from the noncoding region (the *rps16* gene intron, the *trnL–trnF* intergenic spacer, and the *atpB–rbcL* spacer) and two from coding genic regions (*rbcL*, *atpB*), with the sixth marker from the *Xdh* nuclear gene. Based on the large number of nucleotide characters from the chloroplast and nuclear regions as well as the high levels of resolution and support from both parsimony and Bayesian analyses, the results are sufficiently robust to justify reclassification of the Rutaceae, with four subfamilies recognized in contrast to the traditional seven. This subfamily classification includes major rearrangements: (1) taxa within the subfamily Aurantioideae Horan. remain the same; (2) subfamily Cneoroideae Webb encompass the subfamilies Spathelioideae Engl. and Dictyolomatoideae Engl., *Harrisonia* R. Br. ex A. Juss. of the Simaroubaceae DC., *Cneorum* L. of the Cneoraceae Vest, and *Ptaeroxylon* Eckl. & Zeyh. of the Ptaeroxylaceae J.-F. Leroy; (3) subfamily Rutoideae Arn. include *Ruta* L. and *Chloroxylon* DC. of subfamily Flindersioideae Luerss.; and (4) subfamily Amyridoideae Link unite *Flindersia* R. Br. of subfamily Flindersioideae with subfamily Toddalioideae K. Koch and taxa previously included in subfamily Rutoideae.

Key words: Amyridoideae, Aurantioideae, Cneoroideae, Rutaceae, Rutoideae.

The Rutaceae Juss. are a large family of aromatic trees, shrubs, and herbs of approximately 2040 species in 150 to 170 genera (Kubitzki et al., 2011). The family has a more or less cosmopolitan distribution, but it is largely centered in the tropics and temperate regions of the Southern Hemisphere, especially Australia and South Africa. The bulk of the family occupies tropical and subtropical, montane or lowland habitats. Small components lie in the north- and south-temperate zones. The subfamilies Aurantioideae Horan. and Flindersioideae Luerss. are native to the Eastern Hemisphere, the subfamilies Spathelioideae Engl. and Dictyolomatoideae Engl. to the Western Hemisphere, and the subfamilies Toddalioideae K. Koch and Rutoideae Arn. to both hemispheres. Approximately one quarter of all species in the Rutaceae occur in the Western Hemisphere.

The Rutaceae is characterized by the following morphological features: the presence of secretory cavities containing aromatic ethereal oils usually scattered throughout the parenchyma and in the pericarp; sieve tubes with S-type plastids; exstipulate leaves; and a superior ovary (Chase et al., 1999). All other characters at this level manifest a bewildering diversity of character states, especially in floral and fruit morphology. Flowers are perfect or unisexual, actinomorphic or zygomorphic; sepals number from two to five and are distinct or connate at the base; petals usually number from two to five or are sometimes lacking and range from a few millimeters to several centimeters long; stamens number from two to 60 or more, and sometimes are reduced to staminodia; filaments are usually distinct but are rarely connate at the base or rarely adherent to adnate to the petals; and the placentation is axile when the ovary is plurilocular, marginal when the gynoecium is apocarpous or parietal when unilocular syncarous. Fruits range among capsules, samaras, schizocarps, drupes, berries, and hesperidia, with endosperm present or lacking. Due to this level of diversity in the above characters, they are not useful at the subfamily level (Chase et al., 1999).

In addition to diverse morphological features, the biochemical networks and pathways of the Rutaceae produce a tremendous spectrum of secondary metabolites and a wide range of resistances to biotic and abiotic stresses. For instance, Price (1963) stated that the Rutaceae are the most chemically versatile of all plant families. These chemical compounds, including limonoids, flavonoids, coumarins, and alkaloids possess antimicrobial activity (Esterhuizen et al., 2006), herbicidal activity (Aliotta et al., 1996), trypanocidal activity (Ambrozin et al., 2004), insecticidal activity (Marr & Tang, 1992; Rajkumar & Jebanesan, 2008), and antimalarial activity (Fernandez et al., 2009).

In the only systematic treatment of the Rutaceae, Engler (1896, 1931) recognized seven subfamilies: Rhabdodendroideae Engl., Aurantioideae, Spathe-

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lioideae, Dictyolomatoideae, Flindersioideae, Rutoideae, and Toddalioideae. He circumscribed these subfamilies primarily by characters of the gynoecium, especially fruit type. Analysis of *rbcL* sequence data by Gadek et al. (1996) placed *Rhabdodendron* Gilg & Pilg. as sister to the caryophyllid clade, a result also found by Fay et al. (1997) and, more recently, Brockington et al. (2009) and supported by biochemical studies of Wolter-Filho et al. (1985). Based on these conclusive results, *Rhabdodendron* is excluded from this study, as is consideration of its subfamily Rhabdodendroideae. Below is a summary of the remaining six subfamilies for the Rutaceae as included in the current analyses, as had been defined by Engler (1896, 1931).

1. Rutaceae subfam. Aurantioideae Horan. In recent gene analyses (Samuel et al., 2001; Araujo et al., 2003; Morton et al., 2003; Bayer et al., 2009; Morton, 2009) all genera of subfamily Aurantioideae comprise a very strongly supported monophyletic group that is defined by a number of morphological, cytological, and chemical synapomorphies (Waterman, 1975; Grieve & Scora, 1980; Samuel et al., 2001; Morton et al., 2003).

2–3. Subfamilies Spathelioideae Engl. and Dictyolomatoideae Engl. subfam. Dictyolomatoideae consist of the South American genus *Dictyoloma* A. Juss. The subfamily Spathelioideae also consist of a single genus, *Spathelia* L., native to the West Indies and South America. Morton et al. (1996), Chase et al. (1999), Gruppo et al. (2008 [excluding *Harrisonia* R. Br. ex A. Juss.]), Razafimandimbison et al. (2010), and Appelhans et al. (2011), found the consistent pairing with strong support of *Dictyoloma* and *Spathelia* within a moderately supported clade that included *Cneorum* L., *Ptaeroxylon* Eckl. & Zeyh., and *Harrisonia*.

4. Subfamily Flindersioideae Luerss. Engler (1896, 1931) assigned two genera to subfamily Flindersioideae, *Flindersia* R. Br. and *Chloroxylon* DC., both native to the Old World tropics. Morton et al. (1996) and Chase et al. (1999), using molecular data, confirmed that these two genera are not members of the Meliaceae Juss., but rather belong to the Rutaceae. The above two authors and Gruppo et al. (2008) recovered a supported relationship for *Flindersia* forming a clade with *Lunasia* Blanco (subfamily Rutoideae) while *Chloroxylon* formed a clade with *Ruta* L. (subfamily Rutoideae), suggesting the non-monophyly of the subfamily.

5–6. Subfamilies Rutoideae Arn. and Toddalioideae K. Koch. The subfamily Rutoideae are the largest of the subfamilies with approximately 100 genera and 1204 species from temperate and tropical regions worldwide. Five tribes are currently recognized (sensu Engler, 1896, 1931): the Boroniinae Bartl. (19 genera), Galipeeae Kallunki (28 genera), Diosmeae DC. (13 genera), Ruteae Juss. (seven genera), and Zanthoxyleae Dumort. (32 genera). Engler (1896, 1931) defined the subfamily Toddalioideae (Dendrologie 1: 564. 1869) as consisting of approximately 21 genera and 152 species, also native to tropical and temperate regions worldwide, as a separate subfamily. However, revisionary studies by Hartley (1974, 1977, 1981, 1982) indicated that those genera of subfamily Toddalioideae with syncarpous, drupaceous fruits (e.g., *Acronychia* J. R. Forst. & G. Forst., *Toddalia* Juss., and *Phellodendron* Rupr.) were more closely related to genera of subfamily Rutoideae with apocarpous or subapocarpous follicular fruits (e.g., *Melicope* J. R. Forst. & G. Forst., *Zanthoxylum* L., and *Tetradium* Lour.). Morton et al. (1996) and Chase et al. (1999) revealed that the subfamily is paraphyletic, with taxa from subfamilies Toddalioideae and Flindersioideae nested within subfamily Rutoideae. Poon et al. (2007) examined 12 genera of the subfamilies Rutoideae and Toddalioideae and found once again that these were not monophyletic groups. This study also showed that the genus *Euodia* J. R. Forst. & G. Forst. was polyphyletic while the monophyly of *Zanthoxylum* was ambiguous. *Tetradium* was closely related to *Phellodendron*, *Toddalia*, and *Zanthoxylum*, and was called the proto-Rutaceae group. Poon et al. (2007) used a limited number of genera and genes in their study, thus much more data are needed before a reliable phylogeny can be established. Elucidation of the phylogeny of Rutaceae and subfamily Rutoideae is important as its members possess unique metabolic pathways with capabilities for the production of diverse and complex Rutalean metabolites that have demonstrated a wide range of known chemical activities with the potential for other valuable activities.

In summary, Morton et al. (1996, 2003), Chase et al. (1999), Bayer et al. (2009), and Morton (2009) concluded the following: (1) that the Simaroubaceae DC. and Meliaceae are the closest relatives of Rutaceae; (2) that subfamily Aurantioideae are monophyletic; (3) that subfamily Flindersioideae belong in the Rutaceae but are not monophyletic, and that the two genera (*Flindersia* and *Chloroxylon*) appear to be related to different genera in the subfamily Rutoideae; (4) that the subfamilies Dictyolomatoideae and Spathelioideae, each monogeneric, are by default monophyletic, but both are part of a clade that includes *Harrisonia*, *Cneorum*, and *Ptaeroxylon*; (5) that subfamily Toddalioideae should be merged with subfamily Rutoideae; and (6) that the Rutaceae, are paraphyletic, and monophyly can be achieved perhaps by the inclusion of *Harrisonia*, *Cneorum*, and *Ptaeroxylon* in a recircumscribed subfamily Spathelioideae. Gruppo et al. (2008) came to the exact same conclusions but added that none of the tribes with more than one genus is monophyletic (except Diosmeae) and that traditionally used characters at the subfamily and tribal level are not useful.

Recent molecular phylogenetic gene studies of the remaining six subfamilies for the Rutaceae include Chase et al. (1999), Scott et al. (2000), Poon et al. (2007), Gruppo et al. (2008), Morton et al. (2003), Bayer et al. (2009), Morton (2009), Razafimandimbison et al. (2010), and Appelhans et al. (2011). These studies have analyzed, at most, 59 genera out of a total of 160 genera of Rutaceae, using 10 chloroplast genes and one nuclear gene. In order to redefine the family and subfamilies, a more complete survey of the Rutaceae genera with a consistent increased use of molecular markers is required.

The current study utilized six genomic regions, including five chloroplast markers and one nuclear gene. This reflects the most comprehensive coverage using molecular data at the family level. Three of the chloroplast genome regions analyzed were from the noncoding region: the *rps16* gene intron, the *trnL–trnF* intergenic spacer, and the *atpB–rbcL* spacer. The intron of the *rps16* is a group II intron that was first used for phylogenetic studies by Oxelman et al. (1997). The *trnL–trnF* region consists of the *trnL* intron and the *trnL–trnF* intergenic spacer (Taberlet et al., 1991). The *atpB–rbcL* spacer lies between the gene for the large subunit of ribulose-1-5-bisphosphate-carboxylase (*rbcL*) and the gene for the beta subunit of the chloroplast ATP-synthase (*atpB*).

Both the *rbcL* and *atpB* genes are located in the large single-copy region of the plastid genome; their coding sequences are on opposite strands separated by an intergenic spacer of approximately 600–800 bp. The genes code for distinct enzymatic functions: *rbcL* codes for the large subunit of the ribulose-1-5 bisphosphate-carboxylase/oxygenase, a soluble enzyme in the stroma that functions in both the light-dependent and light-independent (Calvin cycle) reactions, whereas *atpB* codes for the beta subunit of the ATP synthase complex, which is bound to the thylakoid membrane and is the site of the light-dependent reactions of photosynthesis. The *atpB* gene is contiguous with the *atpE* gene and downstream from the *rbcL* gene (Zurawski et al., 1982).

Xanthine dehydrogenase (*Xdh*), the nuclear gene, is a member of the molybdenum (Mo) hydroxylase family of enzymes that play important metabolic roles in purine metabolism and plant hormone biosynthesis (Mendel & Hansch, 2002). Among Mo-enzymes, the alignment of amino acid sequences permits domains that are well conserved to be defined although highly variable. This gene is located on chromosome 4, contig fragment 82 (Morton, pers. obs.). The *Xdh* gene is approximately 4262 bp long, and the sequenced segment lies from 363 to 1719 bps. This segment contains approximately four indel regions. To date, only one copy has been reported, except in silkworms and *Arabidopsis* Heynh., in which a second copy has been examined (Hesberg et al., 2004).

Materials and Methods

SELECTION OF TAXA

Thirty-four species representing all subfamilies and tribes of Rutaceae were included in the analysis. Similar exemplar species of Rutaceae, which were used in the *rbcL* and *atpB* analysis of Chase et al. (1999), have been used in this study. Representatives of families that have been suggested as relatives of Rutaceae (Fernando & Quinn, 1995; Chase et al., 1999) have also been included, e.g., the Cneoraceae, Meliaceae, Ptaeroxylaceae, and Simaroubaceae s. str.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Total genomic DNA was isolated from fresh or desiccant-dried leaf tissue or from herbarium material. DNA preparation either followed the same procedures as Chase et al. (1999), or by extraction with the Plant DNeasy Mini Kit (Qiagen, Clifton Hill, Victoria, Australia). The 50-µl (chloroplast DNA; cpDNA) and 25-µl (nuclear ribosomal DNA; nrDNA) volume reactions were constituted in a buffer of 20 mM Tris HCl (pH 8.4) buffer, and 50 mM KCl and utilized, 3 mM $MgCl_2$, 200 uM dNTPs, 1 uM of each primer, and 1.25 U (cpDNA) and 0.75 U (nrDNA) of Taq polymerase (Invitrogen, Carlsbad, California, U.S.A.). With the reactions on ice, 100–500 ng of template total DNA was added, and then tubes were placed in the thermal cycler (Biometra, Goettingen, Germany). Primers and cycle reactions for the six markers are reported in Table 1.

The PCR products were cleaned using the QIAquick PCR purification kit (QIAGEN Inc., Chatsworth, California, U.S.A.), following the manufacturer's protocols and eluting with double distillated water (Millipore, Billerica, Massachusetts, U.S.A.). Clean products were directly sequenced, using the ABI PRISM Dye Terminator Cycle Sequencing Ready Kit with AmpliTaq DNA Polymerase (Applied Biosystems Inc., Foster City,

Table 1. Primers and PCR protocols.

DNA region	Primer name	Reference	Initial denaturing	Cycles	Denaturing	Primer annealing	Extension	Final extension
rbcL	1F	Olmstead et al., 1992	—	35	1 min. at 95°C	30 sec. at 50°C	1 min. at 72°C	
	1460R							
	636F							
	724R							
atpB	2F	Hoot et al., 1995	—	35	1 min. at 95°C	30 sec. at 50°C	1 min. at 72°C	
	1492R							
	611F							
	766R							
atpB–rbcL spacer	520F	Crayn & Quinn, 2000	—	35	1 min. at 95°C	30 sec. at 50°C	1 min. at 72°C	7 min. at 72°C
	3158R							
rps16	rpsF	Oxelman et al., 1997	—	25	30 sec. at 95°C	50 sec. at 57°C	2 min. at 72°C	
	rpsR2							
trnL–trnF	trn-c	Taberlet et al., 1991	7 min. at 94°C	30	1 min. at 94°C	1 min. at 45°C	1 min. at 72°C	7 min. at 72°C
	trn-d							
	trn-e							
	trn-f							
Xdh	486F	Morton, 2011	1 min. at 96°C	34	30–60 sec. at 94°C	1 min. at 48°C–60°C	1 min. at 68°C–72°C	7 min. at 72°C
	1365R							
	1296F							
	1869R							

Table 2. GenBank accession numbers for molecular data sets from five chloroplast markers, three from the noncoding region (the *rps16* gene intron, the *trnL–trnF* intergenic spacer, and the *atpB–rbcL* spacer) and two from coding genic regions (*rbcL*, *atpB*), as well as a sixth marker from the *Xdh* nuclear gene. Newly sequenced taxa for this study are in bold with voucher information. All remaining GenBank accession numbers are from previous studies, as indicated by footnotes. Collection abbreviations are M. W. Chase (MWC) and C. M. Morton (CMM). NA, not applicable. References indicated by subscript after number and papers listed below.

	Voucher	*rbcL*	*atpB*	*atpB–rbcL* spacer	*trnL*	*rps16*	*Xdh*
Adenandra uniflora (L.) Willd.	South Africa. *MWC 1769*, K	AF066803^{a}	AF066832^{a}	AM502754 (*A. villosa* Eckl. & Zeyh.)^{p}	JX307298	JX307327	JX307301
Aegle marmelos (L.) Corrêa	Indonesia. *MWC 1340*, K	AF066811^{a}	AF066839^{a}	FJ434197^{g}	AY295294^{h}	AY295268^{h}	JX307302
Atalantia ceylanica Oliv.	Indonesia. *MWC 1341*, K	AF066812^{a}	AF066840^{a}	FJ434186^{g}	AY295288^{h}	AY295262^{h}	JX307303
Calodendrum capense Thunb.	South Africa. *MWC 1821*, K	AF066805^{a}	AF066834^{a}	JX307335	AF025511^{k}	JX307328	JX307304
Casimiroa edulis La Llave & Lex.	NA	AF066808^{a}	AF066837^{a}	EF126506^{f}	EU853782 (*C. tetrameria* Millsp.)^{j}	EF126573^{f}	EU264403^{q}
Chloroxylon swietenia DC.	Indonesia. *MWC 1291*, K	AF066802^{a}	AF066831^{a}	FJ434177^{g}	AY295276^{h}	AY295250^{h}	JX307305
Choisya mollis Standl.	United States. *MWC 1755*, K	AF066800^{a}	AF066829^{a}	EF126507 (*C. ternate* Kunth)^{f}	EU853784^{j}	EU853730^{j}	JX307306
Chorilaena quercifolia Endl.	Australia. *M. Richardson s.n.*, CBG	AF066810^{a}	AF066838^{a}	JX307336	EU853785^{j}	EU853731^{j}	JX307307
Clausena excavata Burm. f.	Indonesia. *MWC 1343*, K	AF066813^{a}	AF066841^{a}	FJ434179^{g}	EF126674^{f}	AY295258^{h}	JX307308
Cneorum pulverulentum Vent.	Spain. *MWC 3428*, K	U38858^{c}	AF066828^{a}	JX307337	EU853787^{j}	EU853733^{j}	JX307309
Correa pulchella J. Mackay ex Sweet	Australia. *MWC 1757*, K	AF066816^{a}	AF066844^{a}	JX307338	EU853790^{j}	EU853736^{j}	JX307310
Dictamnus albus L.	Australia. *MWC 1820*, K	AF066801^{a}	AF066830^{a}	JX307339	EU853792^{j}	EU853738^{j}	JX307311
Dictyoloma vandellianum A. Juss.	Brazil. *Pirani et al. 3039*, K	AF066823^{a}	AF066853^{a}	JX307340	EU853793^{j}	EU853739^{j}	JX307312
Diplolaena dampieri Desf.	Australia. *M. Richardson s.n.*, CBG	AF066807^{a}	AF066836^{a}	JX307341	EU853794^{j}	EU853740^{j}	JX307313
Eremocitrus glauca (Lindl.) Swingle	NA	AF066819^{a}	AF066847^{a}	FJ434188^{g}	AY295293^{h}	AY295267^{h}	EU264414^{q}
Eriostemon angustifolius Paul G. Wilson	Australia. *MWC 2005*, K	AF156883 (*E. brevifolius* A. Cunn. ex Endl)^{a}	AF156882 (*E. brevifolius* A. Cunn. ex Endl)^{a}	JX307342	JX307299	JX307329	JX307314
Flindersia australis R. Br.	Australia. *I. R. Telford 11032*, CBG	U38861^{c}	EF118872^{f}	JX307343	EF126677^{f}	EF126610^{f}	JX307315
Fortunella polyandra (Ridl.) Tanaka	NA	AF066799 (*Fortunella japonica* Swingle)^{a}	AF066827 (*Fortunella* sp. Swingle)^{a}	FJ434187^{g}	AY295291^{h}	AF320273 (*F. hindsii* Swingle)^{h}	EU264397^{q}

Table 2. Continued.

	Voucher	*rbcL*	*atpB*	*atpB–rbcL* spacer	*trnL*	*rps16*	*Xdh*
Glycosmis pentaphylla (Retz.) DC.	Indonesia. *MWC 1345*, K	AF066820[a]	AF066849[a]	FJ434178[g]	AY295279[h]	AY295253[h]	JX307316
Harrisonia perforata (Blanco) Merr.	Indonesia. *CMM 1996-10*, CM	U38863[c]	JX307295	JX307344	EU721390 (*H. abyssinica* Oliv.)[r]	—	JX307317
Lunasia amara Blanco	Indonesia. *MWC 1347*, K	AF066814[a]	AF066842[a]	JX307345	EU853805[j]	EU853753[j]	—
Melicope ternata J. R. Forst. & G. Forst.	Australia. *MWC 1759*, K	AF116271[a]	AF066826[a]	JX307346	EU852808[j]	EU853756[j]	EU264401[q]
Murraya paniculata (L.) Jack	cult. United States. *CMM 2012-1*, CM	EF119891[a]	JX307296	FJ434180[g]	EU853810[j]	AF320268[o]	—
Phebalium woombye (Bailey) Domin	Australia. *H. Fernanado 1402*, CBG	AF066822[a]	AF066852[a]	JX307347	JX307300	—	JX307318
Phellodendron amurense Rupr.	cult. Australia. *MWC 1771*, K	AF066804[a]	AF066833[a]	JX307348	DQ225993[l]	JX307330	JX307319
Pilocarpus pennatifolius Lem.	cult. Australia. *MWC 1760*, K	AF066809[a]	AF066825[a]	JX307349	EU853811 (*P. spicatus* A. St.-Hil.)[j]	EU853761 (*P. spicatus* A. St.-Hil.)[j]	EU264408[q]
Poncirus trifoliata (L.) Raf.	NA	AJ235806[b]	AJ238409[a]	FJ434181[g]	AY295282[h]	AY295256[h]	EU264398[q]
Ptaeroxylon obliquum Radlk.	cult. South Africa. *Baker s.n.*, BOL	AF123276[d]	AF066848[a]	JX307350	EU853812[j]	EU853762[j]	JX307320
Ruta graveolens L.	NA	RGU39281[d]	AF035913[a]	FJ434173[g]	AY295275[h]	AY295249[h]	EU264412[q]
Ravenia infelix Vell.	Brazil. *J. Kallunki et al. 614*, NY	JX307294	JX307297	JX307351	EU853814[j]	EU853764[j]	EU264411[q]
Sarcomelicope simplicifolia (Endl.) T. G. Hartley	Australia. *MWC 1762*, K	AF066817[a]	AF066845[a]	JX307352	EU853816[j]	EU853766[j]	JX307321
Severinia buxifolia (Poir.) Ten.	cult. United States. *CMM 2012-2*, CM	AF066806[a]	AF066835[a]	FJ434183[g]	EU369566[i]	AY295264[h]	JX307322
Skimmia anquetilia N. P. Taylor & Airy Shaw	cult. Australia. *MWC 1766*, K	AF066818[a]	AF066846[a]	EF126564[f]	EU853819 (*S. japonica* Thunb.)[j]	EF126630[f]	JX307323
Spathelia excelsa (K. Krause) R. S. Cowan & Brizicky	NA	AF066798[a]	AF066854[a]	—	EU853820[j]	EU853770[j]	EU264413[q]
Zanthoxylum monophyllum (Lam.) P. Wilson	NA	U39282[a]	AF035919[a]	EF362484[f]	EU853773 (*Z. rhoifolium* Lam.)[j]	EF126633[f]	EU264402[q]
Zanthoxylum fagara (L.) Sarg.	Mexico. *Henrickson 22590*, CM	AF066815[d]	AF066843[a]	EF126549[f]	EF126684[f]	JX307331	JX307324

Table 2. Continued.

	Voucher	*rbcL*	*atpB*	*atpB–rbcL* spacer	*trnL*	*rps16*	*Xdh*
Outgroup Taxa							
Ailanthus altissima (Mill.) Swingle	United States. *H. George s.n.*, CM	L12566[c]	AF035895[a]	JX307353	FN599505	JX307332	EU264405[q]
Trichilia emetica Vahl	Unknown. *MWC 552*, K	U39082[d]	AF066851[a]	JX307354	FJ039160 (*T. surinamensis* C. DC.)[a]	JX307333	JX307325
Simaba bidwillii (Hook. f.) Feuillet	Australia. *Fernando et al. 1995*, QRS	U38926[c]	AF066824 (*S. baileyana* (Oliv.) Feuillet)[a]	JX307355	EU853818 (*S. cedron* Planch.)[j]	EU853768 (*S. cedron* Planch.)[j]	JX307326
Swietenia macrophylla King	cult. United States. *MWC 250*, NCU	U39080[d]	AF066857[a]	JX307356	EF489262[m]	JX307300	EU264415[q]

[a] Chase et al., 1999.
[b] Chase et al., 1993.
[c] Fernando & Quinn, 1995.
[d] Gadek et al., 1996.
[e] Gadek et al., 1992.
[f] Bayer et al., 2009.
[g] Morton, 2009.
[h] Morton et al., 2003.
[i] Li et al., 2007.
[j] Gruppo et al., 2008.
[k] Scott et al., 2000.
[l] Poon et al., 2007.
[m] Salvo et al., 2008.
[n] Buerki et al., 2009.
[o] Samuel et al., 2001.
[p] Trinder-Smith et al., 2007.
[q] Morton, 2011.
[r] Buerki et al., 2009.

California, U.S.A.). Unincorporated dye terminators were removed using the DyeEx dye-terminator removal system (QIAGEN Inc.), following the manufacturer's recommendations. Samples were then loaded into an ABI 3100 DNA Sequencer (ABI, Grand Island, New York, U.S.A.). The sequencing data were analyzed and edited using the Sequencher software program (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.).

PHYLOGENETIC ANALYSIS

Boundaries of the above gene regions were determined by comparison with sequences in GenBank. The following alignment criteria and methodology were used: (1) when two or more gaps were not identical but overlapping, they were scored as two separate events; and (2) phylogenetically informative indels (variable in two or more taxa) were scored as one event at the end of the dataset. All DNA sequences reported in the analyses have been deposited in GenBank (Table 2).

For phylogenetic analysis, the data sets were examined using maximum parsimony and Bayesian analyses. Maximum parsimony analyses used the heuristic search option in PAUP* 4.0b8 (Swofford, 2002), with uninformative characters excluded. Searches were conducted with 1000 random-taxon-addition replicates with TBR branch swapping, steepest descent and MulTrees selected, and with all characters and states weighted equally and unordered. All trees from the replicates were then swapped onto completion, all shortest trees were saved, and a consensus tree was computed. Relative support for individual clades was estimated with the bootstrap method (Felsenstein, 1985). One thousand pseudoreplicates were performed with uninformative characters excluded. Ten random-taxon-addition heuristic searches for each pseudoreplicate were performed, and all minimum-length trees were saved for each search. To reduce bootstrap search times, branches were collapsed if their minimum length was zero ("amb-").

Bayesian analyses were performed using MrBayes 3.1.1 (Ronquist et al., 2005) on the combined molecular datasets. The substitution model for each DNA region was selected with MrModeltest 2.3 (Nylander, 2004) under the Akaike information criterion (AIC). The parameters for the Bayesian analyses were as follows: nst = 6; rates = Invgamma; set autoclose = yes; mcmcp ngnen = 1000000; prinfewq = 100; samplefreq = 10; savebriens = yes. The first 25% of the trees were regarded as "burn in" and omitted, and the majority rule consensus tree was obtained in PAUP* from the remaining trees.

To determine the combinability of the two datasets, their data structures were compared using methods outlined by Mason-Gamer and Kellogg (1996), who discussed various ways to assess conflict between datasets. In one method the combination of independent datasets is possible if the trees do not conflict or if conflict receives low bootstrap support. Therefore, each node on the independent trees is tested for congruence against the other. If the nodes do not contain conflicting information, they are congruent and the datasets are combinable. Where there are incongruent nodes, the bootstrap values for each node are examined. If the support is less than 75%, then there is no hard conflict and the incongruence is interpreted as being due to chance. In this study the different datasets were also analyzed in combination to see how each dataset confirmed or differed from the tree topologies from the other datasets and to adopt a hypothesis of phylogenetic relationships for the subfamilies.

RESULTS

The inclusion of gap coding in all datasets containing molecular data resulted in more homoplasy and lack of resolution; therefore, gap coding was not used in the following results. GenBank sequences JX307295–JX307356 were generated specifically for this study (Table 2).

RBCL

No gaps were required for proper alignment of the 40 *rbcL* sequences. Mean percentage G + C content is 45%. Of the 1410 positions constituting the aligned *rbcL* sequences, 307 (22%) were variable and 157 (11%) were parsimony-informative. The analysis recovered 18,352 equally optimal trees of 406 steps (CI = 0.50, RI = 0.68; cf. Fig. 1).

The Rutaceae s.l. were supported as monophyletic in the strict consensus tree. Basal within the majority rule tree, the Rutaceae s.l. consisted of two main clades, the weakly supported clade comprised by the (*Cneorum pulverulentum* Vent, Cneoraceae + *Ptaeroxylon obliquum* Radlk., Ptaeroxylaceae; BS 89%) clade sister to the ((*Dictyoloma vandellianum* A. Juss. + *Spathelia excelsa* (K. Krause) R. S. Cowan & Brizicky; BS 97%) + *Harrisonia perforata* (Blanco) Merr., Simaroubaceae) clade, and a moderately supported major clade comprising the rest of the family (BS 89%). Within the largest clade, three pairs of genera were supported with bootstrap values greater than 75%; these are, starting from the base: (1) the strongly supported (BS 100%) pair composed by *Severinia buxifolia* (Poir.) Ten. and *Atalantia*

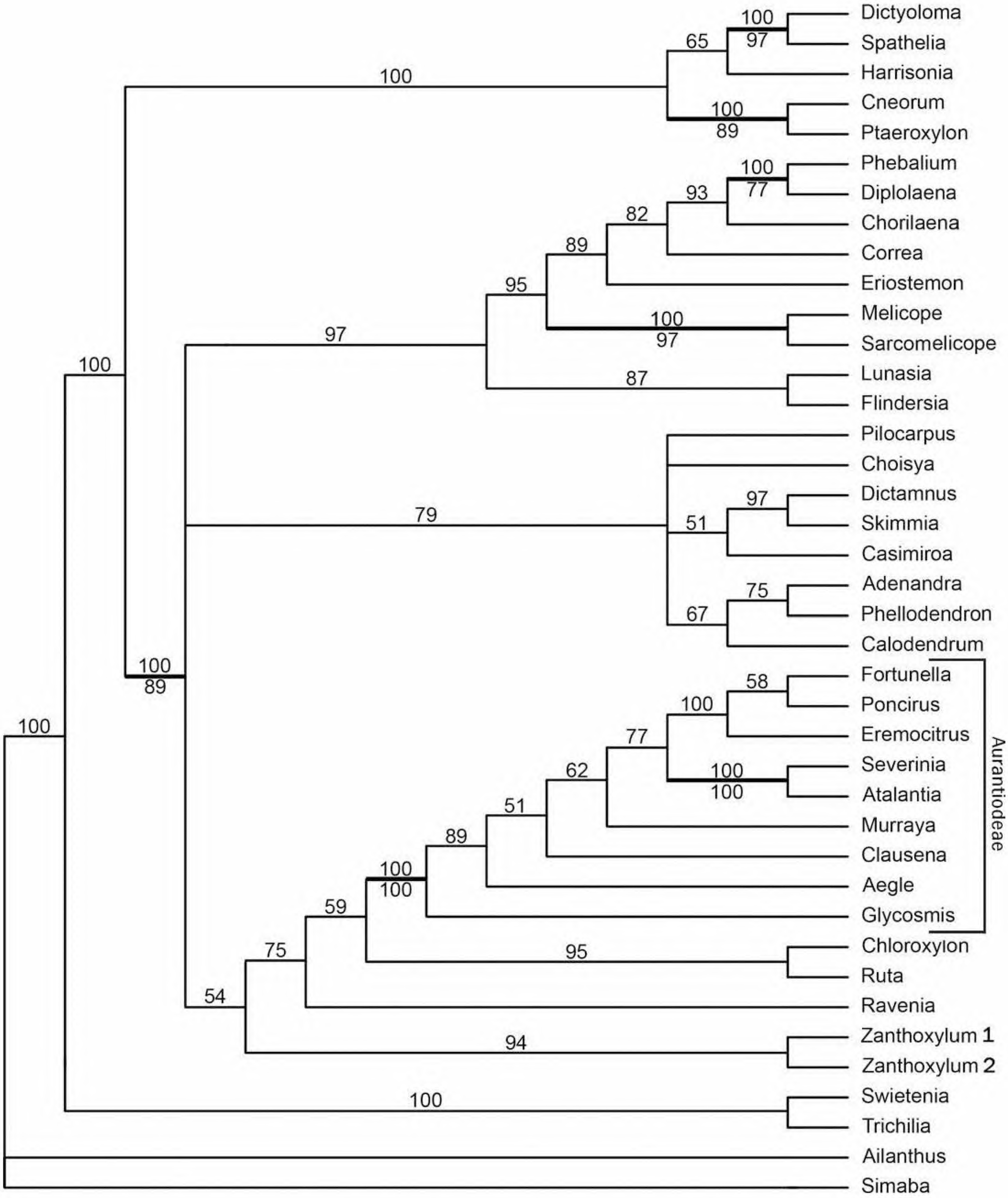

Figure 1. The majority rule consensus tree of 18,352 most-parsimonious trees (length = 406 steps, CI = 0.50, RI = 0.68) obtained from the *rbcL* data from the Rutaceae. The bracket indicates the taxa belonging to subfamily Aurantioideae. Numbers above the branches indicate consensus values; numbers below the branches are bootstrap values for nodes with more than 75% support.

ceylanica Oliv., which was included in the well-supported Aurantioideae clade (BS 100%; Fig. 1); (2) the clade comprising *Melicope ternata* J. R. Forst. & G. Forst. and *Sarcomelicope simplicifolia* (Endl.) T. G. Hartley (BS 97%); and (3) the clade consisting of *Phebalium woombye* (Bailey) Domin and *Diplolaena dampieri* Desf. (BS 77%).

ATP*B*

No gaps were required for proper alignment of the 40 *atpB* sequences. Mean percentage G + C content is 44%. Of the 1489 positions constituting the aligned *atpB* sequences, 292 (20%) were variable and 153 (10%) were parsimony-informative. The

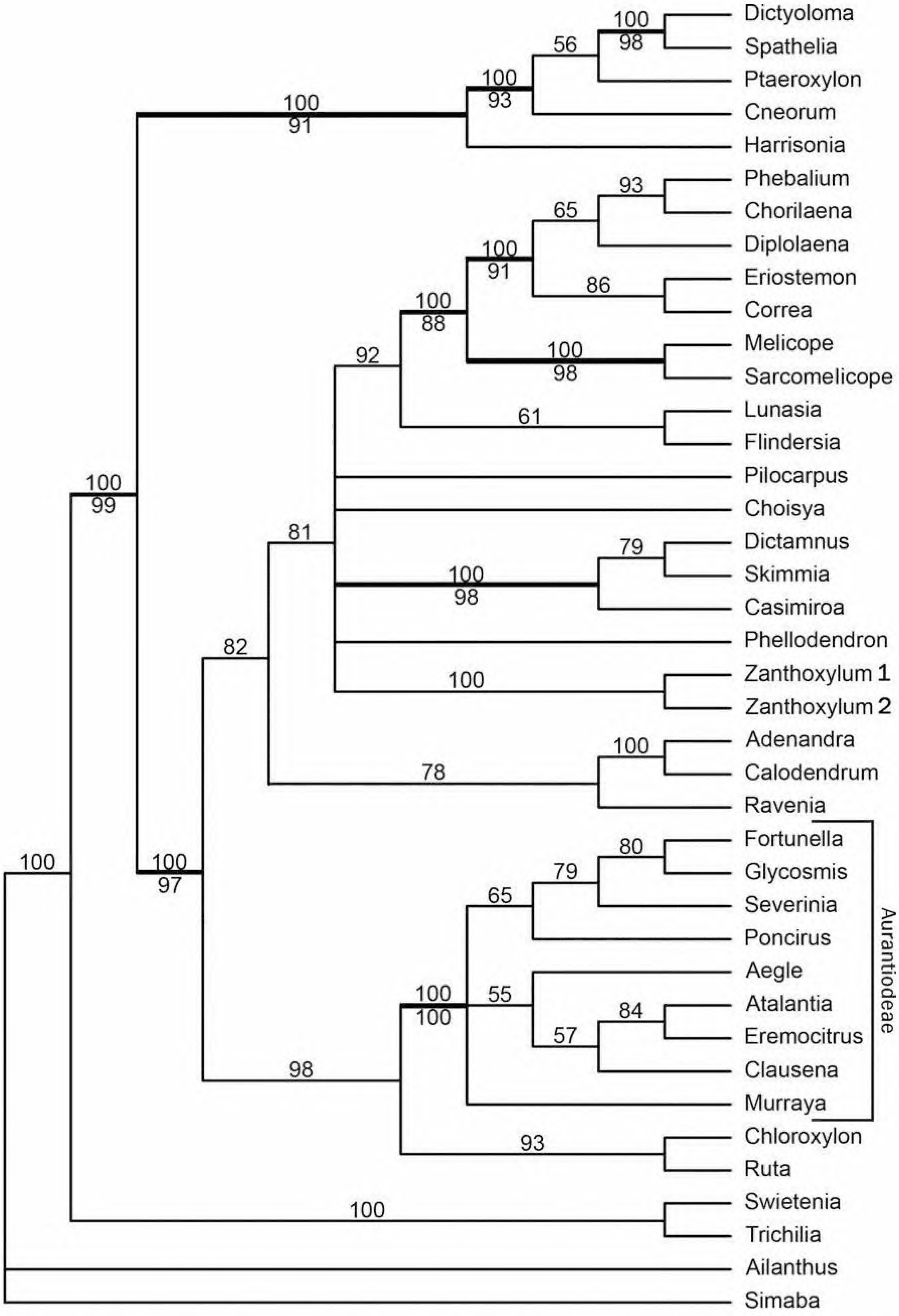

Figure 2. The majority rule consensus tree of 9763 most-parsimonious trees (length = 353 steps, CI = 0.53, RI = 0.72) obtained from the *atpB* data from the Rutaceae. The brackets indicate the taxa belonging to subfamily Aurantioideae. Numbers above the branches indicate consensus values; numbers below the branches are bootstrap values for nodes with more than 75% support.

analysis recovered 9763 equally optimal trees of 353 steps (CI = 0.53, RI = 0.72; cf. Fig. 2).

The Rutaceae s.l. were supported as monophyletic in the strict consensus tree (BS 99%). Basal within the majority rule tree, the Rutaceae s.l. clade was a clade (BS 91%) comprised of *Harrisonia perforata*, Simaroubaceae sister to the clade ((*Dictyoloma vandellianum* + *Spathelia excelsa*; BS 98%) + *Ptaeroxylon obliquum*, Ptaeroxylaceae + *Cneorum pulverulentum*, Cneoraceae; BS 93%). The rest of the family exemplars (BS 97%) consisted of small clades. Clades that contained bootstrap support greater than 75%, starting from the base, include: (1) a clade containing members of the subfamily Aurantioideae

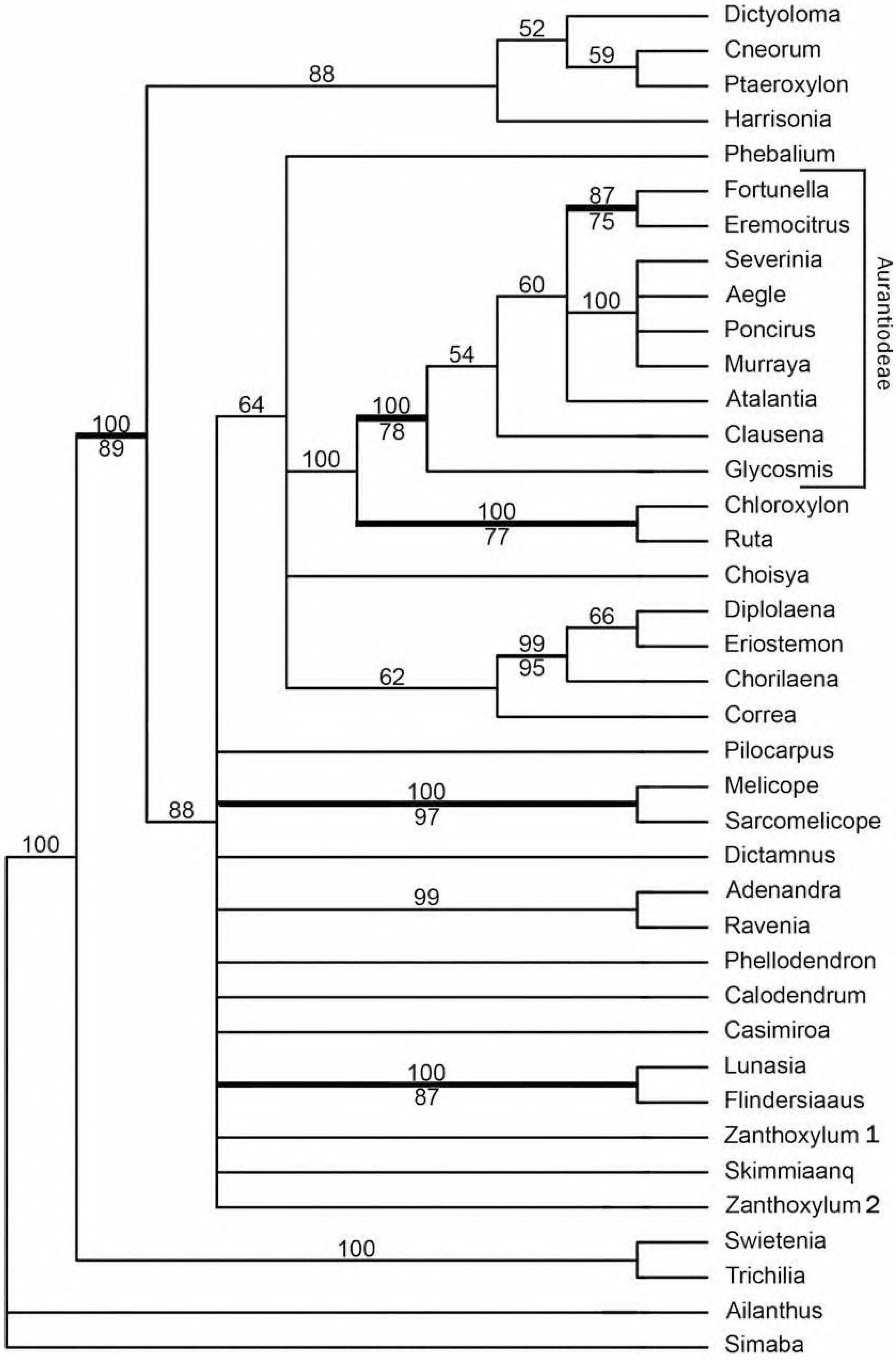

Figure 3. The majority rule consensus tree of 73,552 most-parsimonious trees (length = 657 steps, CI = 0.53, RI = 0.65) obtained from the *atpB–rbcL* spacer data from the Rutaceae. The bracket indicates the taxa belonging to subfamily Aurantioideae. Numbers above the branches indicate consensus values; numbers below the branches are bootstrap values for nodes with more than 75% support.

(BS 100%); (2) a clade containing (*Casimiroa edulis* La Llave & Lex, *Dictamnus albus* L., and *Skimmia anquetilia* N. P. Taylor & Airy Shaw; BS 98%); and (3) a clade (BS 88%) containing ((*Melicope ternata* + *Sarcomelicope simplicifolia*; BS 98%) sister to a clade containing (*Phebalium woombye*, *Chorilaena quercifolia* Endl., *Diplolaena dampieri*, *Eriostemon angustifolius* Paul G. Wilson, and *Correa pulchella* J. Mackay ex Sweet; BS 91%)).

RBC*L*–ATP*B* SPACER

Approximately 16 gaps were required for proper alignment of the 39 sequences (*Spathelia* not

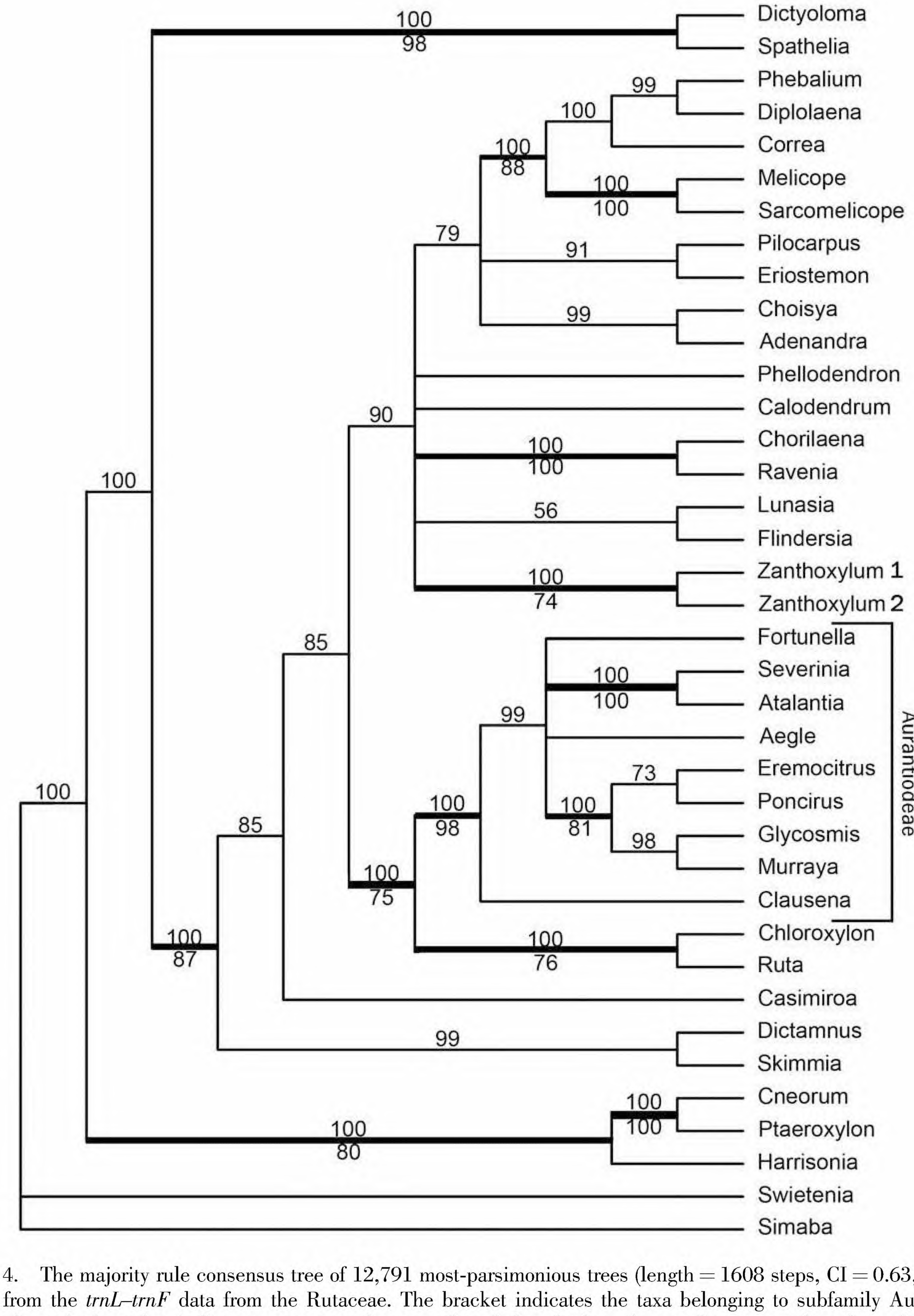

Figure 4. The majority rule consensus tree of 12,791 most-parsimonious trees (length = 1608 steps, CI = 0.63, RI = 0.53) obtained from the *trnL–trnF* data from the Rutaceae. The bracket indicates the taxa belonging to subfamily Aurantioideae. Numbers above the branches indicate consensus values; numbers below the branches are bootstrap values for nodes with more than 75% support.

sequenced) for *rbcL–atpB* spacer. These gaps ranged from one to 29 bps. None of these gaps were parsimony-informative. Mean percentage G + C content is 34%. Of the 987 positions constituting the aligned sequences, 452 (46%) were variable and 222 (23%) were parsimony-informative. The analysis recovered 73,552 equally optimal trees of 657 steps (CI = 0.53, RI = 0.65; cf. Fig. 3).

The Rutaceae s.l. was supported as a monophyletic clade in the strict consensus tree (BS 89%). Most of the Rutaceae consisted of a polytomy in the majority rule tree except for several small clades with bootstrap support greater than 75%, which included: (1) the (*Lunasia amara* Blanco + *Flindersia australis* R. Br.; BS 87%) clade; (2) the (*Melicope ternata* + *Sarcomelicope simplicifolia*; BS 97%) clade; (3) the

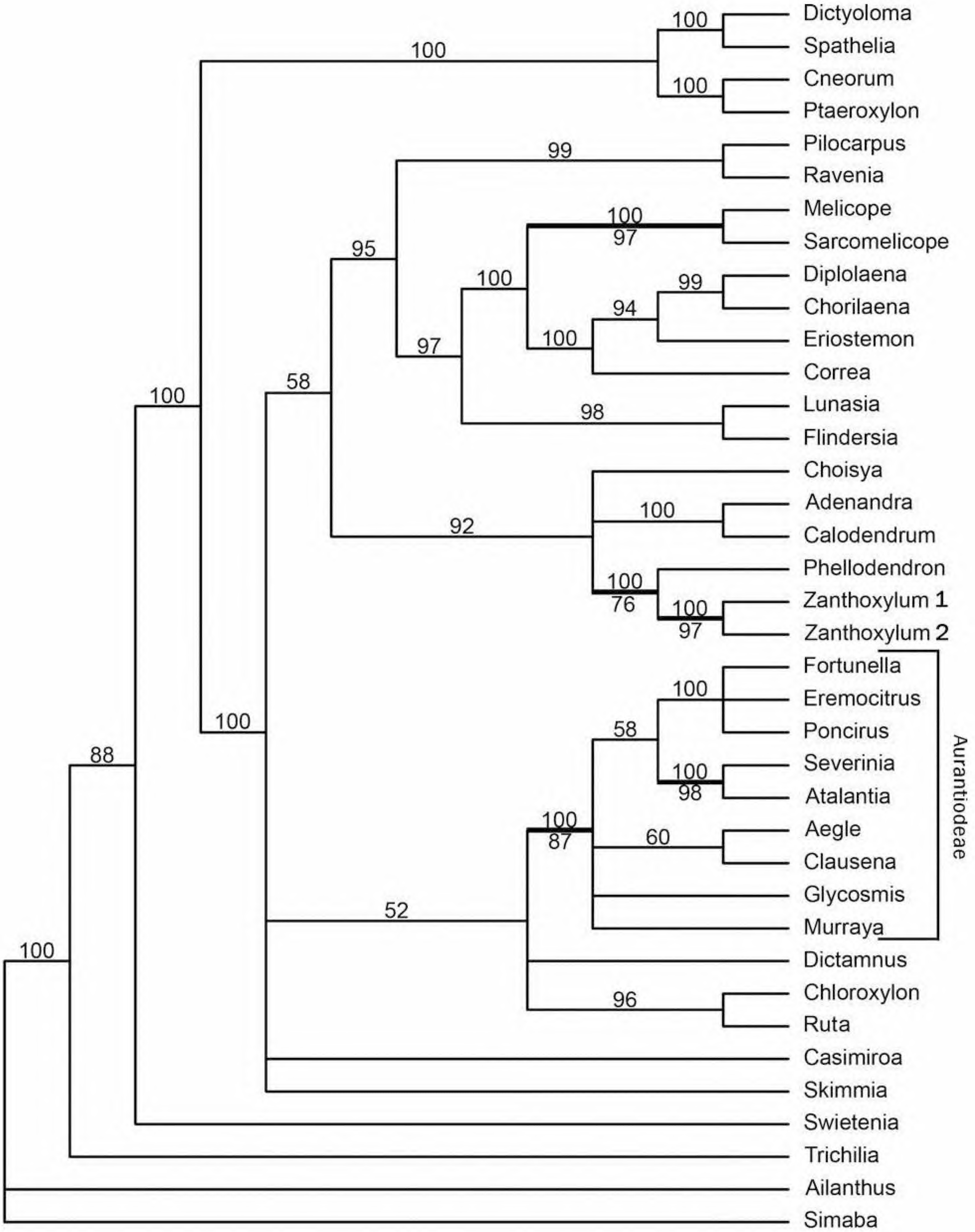

Figure 5. The majority rule consensus tree of 2172 most-parsimonious trees (length = 1643 steps, CI = 0.59, RI = 0.52) obtained from the *rps16* data from the Rutaceae. The bracket indicates the taxa belonging to subfamily Aurantioideae. Numbers above the branches indicate consensus values; numbers below the branches are bootstrap values for nodes with more than 75% support.

clade (*Diplolaena dampieri*, *Eriostemon angustifolius* + *Chorilaena quercifolia*; BS 95%); (4) the clade containing members of the subfamily Aurantioideae (BS 78%) with the (*Fortunella polyandra* (Ridl.) Tanaka + *Eremocitrus glauca* (Lindl.) Swingle; BS 75%) clade internally supported; and (5) the clade (*Chloroxylon swietenia* DC. + *Ruta graveolens* L.; BS 77%).

TRNL–TRNF

Approximately 29 gaps were required for proper alignment of the 39 sequences (*Ailanthus* Desf. not sequenced) for *trnL–trnF*. These gaps ranged from one to 41 bps. None of these gaps were parsimony-informative. Mean percentage G + C content is 36%. Of the 1039 positions constituting the aligned sequences, 612 (60%) were variable and 427 (40%)

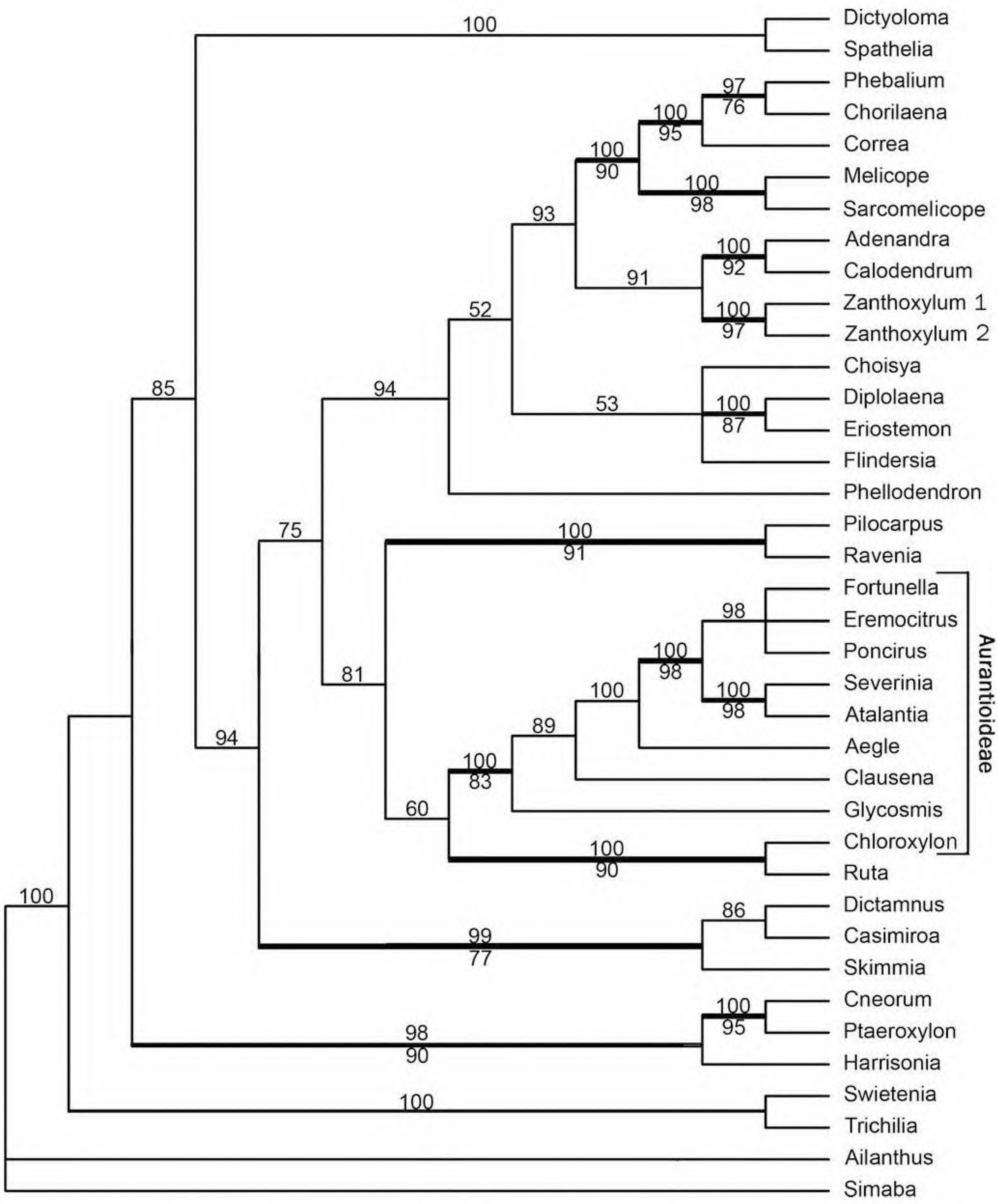

Figure 6. The majority rule consensus tree of 936 most-parsimonious trees (length = 1863 steps, CI = 0.59, RI = 0.58) obtained from the *Xdh* data from the Rutaceae. The bracket indicates the taxa belonging to subfamily Aurantioideae. Numbers above the branches indicate consensus values; numbers below the branches are bootstrap values for nodes with more than 75% support.

were parsimony-informative. The analysis recovered 1654 equally optimal trees of 1380 steps (CI = 0.56, RI = 0.59; cf. Fig. 4 majority rule tree).

The Rutaceae s.l. were monophyletic but not supported in the strict consensus. Basal within the majority rule tree, the Rutaceae s.l. clade was the clade ((*Cneorum pulverulentum*, Cneoraceae + *Ptaeroxylon obliquum*, Ptaeroxylaceae; BS 100%) + *Harrisonia perforata*, Simaroubaceae; BS 80%). The next well-supported clade was (*Dictyoloma vandellianum* + *Spathelia excelsa*; BS 98%). The rest of the family (BS 87%) consisted of small clades that contain bootstrap support greater than 75%. Starting from the base of the clade, these included: (1) a clade containing members of the subfamily Aurantioideae (BS 98%) with (*Severinia buxifolia* + *Atalantia ceylanica*; BS 100%) and (*Eremocitrus glauca*, *Poncirus trifoliata* (L.) Raf., and *Glycosmis penta-*

phylla (Retz.) DC., *Murraya paniculata* (L.) Jack; BS 81%) internally supported; (2) a clade consisting of (*Chloroxylon swietenia* + *Ruta graveolens*; BS 76%) which is sister to the subfamily Aurantioideae clade (BS 75%); (3) a clade containing two species of *Zanthoxylum* (BS 74%); and (4) a clade containing ((*Melicope ternata* + *Sarcomelicope simplicifolia*; BS 100%) sister to a clade containing *Phebalium woombye*, *Diplolaena dampieri*, and *Correa pulchella* (BS 88%)).

RPS16

Approximately 36 gaps were required for proper alignment of the 38 sequences (*Phebalium* Vent. and *Harrisonia* not sequenced) of *rps16*. These gaps ranged from one to 14 bps. None of these gaps were parsimony-informative. Mean percentage G + C content is 34%. Of the 1054 positions constituting the aligned 38 sequences, 777 (74%) were variable and 479 (45%) were parsimony-informative. The analysis recovered 2172 equally optimal trees of 1643 steps (CI = 0.59, RI = 0.52; cf. Fig. 5).

The Rutaceae s.l. were monophyletic in the strict consensus tree. Within the majority rule tree, only five clades contained bootstrap support greater than 75%: (1) the clade containing exemplars of subfamily Aurantioideae (BS 87%); (2) the (*Severinia buxifolia* + *Atalantia ceylanica*; BS 98%) clade; (3) the clade containing two species of *Zanthoxylum* (BS 97%) sister to *Phellodendron amurense* Rupr. (BS 76%); and (4) the (*Melicope ternata* + *Sarcomelicope simplicifolia*; BS 97%) clade.

XDH

Approximately four gaps were required for proper alignment of the 38 sequences (*Murraya* J. Koenig ex L. and *Lunasia* not sequenced). These gaps ranged from one to 11 bps. None of these gaps were parsimony-informative. Mean percentage G + C content was 40%. Of the 1274 positions constituting the aligned 38 sequences, 747 (59%) were variable and 462 (36%) were parsimony-informative. The analysis recovered 936 equally optimal trees of 1863 steps (CI = 0.59, RI = 0.58; cf. Fig. 6).

The Rutaceae s.l. were monophyletic in the majority rule consensus tree. Within the tree, a number of clades contained bootstrap support greater than 75%, which include: (1) the (*Cneorum pulverulentum*, Cneoraceae + *Ptaeroxylon obliquum*, Ptaeroxylaceae; BS 95%) clade sister to *Harrisonia perforata*, Simaroubaceae (BS 90%); (2) the (*Dictamnus albus*, *Casimiroa edulis*, and *Skimmia anquetilia*; BS 77%) clade; (3) the clade containing members of the subfamily Aurantioideae (BS 83%) ((*Severinia buxifolia* + *Atalantia ceylanica*; BS 98%) clade sister to (*Fortunella polyandra*, *Eremocitrus glauca*, and *Poncirus trifoliata*; BS 98%); (4) the clade containing (*Chloroxylon swietenia* + *Ruta graveolens*; BS 90%); (5) the (*Pilocarpus pennatifolius* Lem. + *Ravenia infelix* Vell.; BS 91%) clade; (6) the (*Diplolaena dampieri* + *Eriostemon angustifolius*; BS 87%) clade; (7) the (*Adenandra uniflora* (L.) Willd. + *Calodendrum capense* Thunb.; BS 92%) clade; (8) the clade containing two species of *Zanthoxylum* (BS 97%); and (9) the well-supported clade (BS 90%) comprised of (*Melicope ternata* + *Sarcomelicope simplicifolia*; BS 98%) sister to the ((*Phebalium woombye* + *Chorilaena quercifolia*; BS 76%) + *Correa pulchella*; BS 95%) clade.

COMBINED ANALYSIS

Following the methods outlined by Mason-Gamer and Kellogg (1996) and recently applied by Eldenäs and Linder (2000), the data sets were considered combinable. Several nodes shared between the independent trees with bootstrap support greater than 75% included: *Cneorum pulverulentum* + *Ptaeroxylon obliquum*; *Dictyoloma vandellianum* + *Spathelia excelsa*; *Melicope ternata* + *Sarcomelicope simplicifolia*; *Severinia buxifolia* + *Atalantia ceylanica*; *Chloroxylon swietenia* + *Ruta graveolens*; the Aurantioideae clade; the clade (*Dictamnus albus*, *Skimmia anquetilia*, and *Casimiroa edulis*); two species of *Zanthoxylum*; and *Adenandra uniflora* + *Calodendrum capense*. Among the trees only one potentially conflicting node exists with bootstrap support greater than 75%, and this was the *Phebalium woombye–Diplolaena dampieri* clade. Among the independent analyses, the grouping of taxa within this clade has varied to include: *Phebalium woombye* + *Diplolaena dampieri* in the *rbcL*; *Phebalium woombye*, *Chorilaena quercifolia*, *Diplolaena dampieri*, *Eriostemon angustifolius*, and *Correa pulchella* in the *atpB*; *Diplolaena dampieri*, *Eriostemon angustifolius*, *Chorilaena quercifolia* in the spacer; *Phebalium woombye*, *Chorilaena quercifolia*, and *Correa pulchella* in the *Xdh*. The conflict appeared to be due to a lack of resolution or missing taxa; therefore, congruence existed between the data sets and a combined analysis was completed.

Multiple sequence alignment from exemplars from the Rutaceae and all outgroups resulted in a matrix of 7240 characters, of which (46%) included at least one accession with a gap. Mean percentage G + C content is 39%. Of the 7240 positions constituting the aligned sequences, 3259 (45%) were variable and

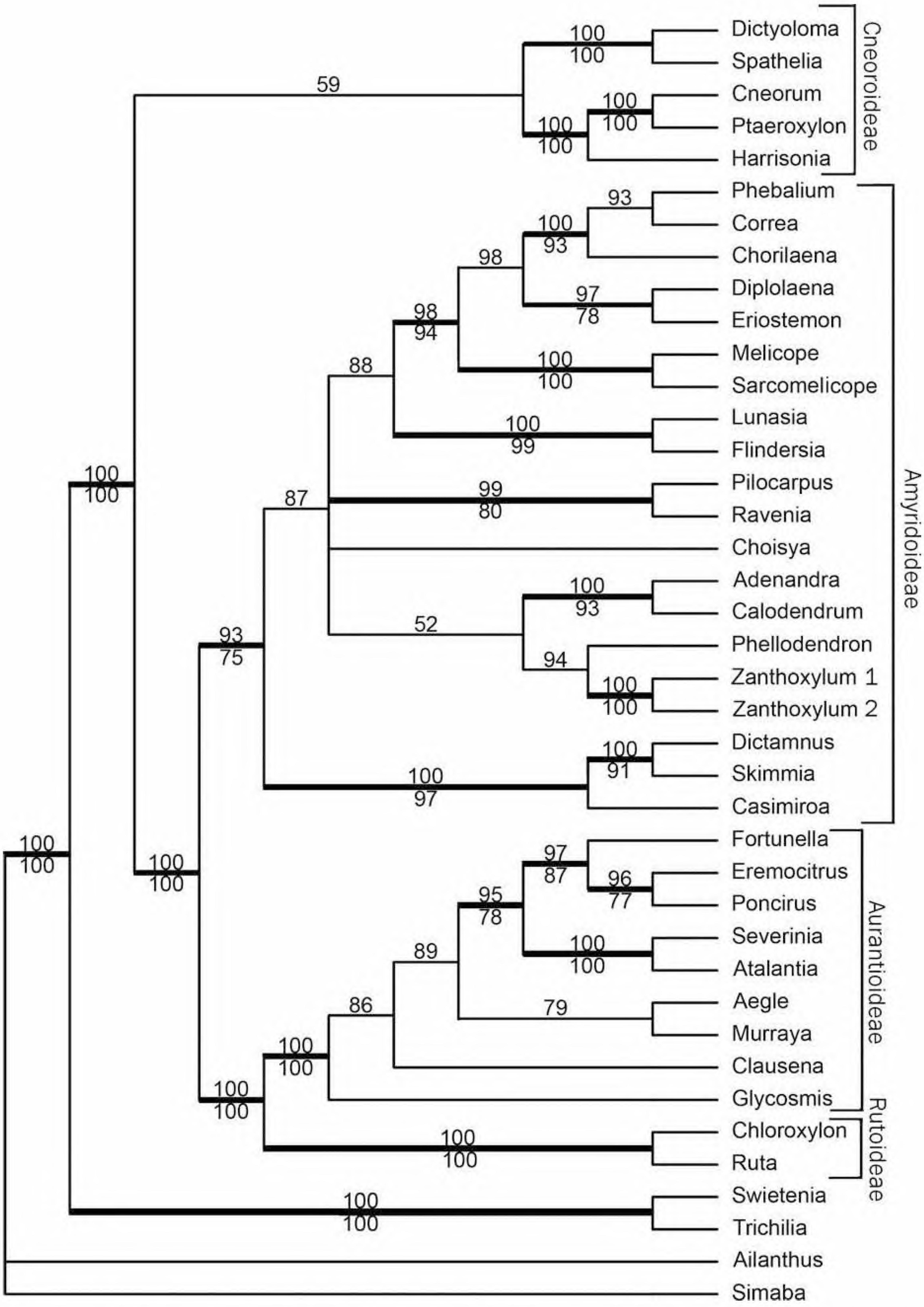

Figure 7. The majority rule consensus tree of 126 most-parsimonious trees (length = 6295 steps, CI = 0.48, RI = 0.54) obtained from the total data from the Rutaceae. Brackets indicate the taxa belonging to the four subfamilies. Numbers above the branches indicate consensus values; numbers below the branches are bootstrap values for nodes with more than 75% support.

1872 (26%) were parsimony-informative. The analysis recovered 126 equally optimal trees of 6295 steps (CI = 0.48, RI = 0.54; cf. Fig. 7).

The Meliaceae were a sister clade to the Rutaceae s.l. (BS 100%). The Rutaceae s.l. were supported as monophyletic in the strict consensus trees. Within the majority rule tree, most clades contain bootstrap support greater than 75%. These include: (1) the strongly supported (BS 100%) clade ((*Cneorum* + *Ptaeroxylon*) + *Harrisonia*); (2) a clade containing (*Dictyoloma vandellianum*, subfamily Dictyolomatoideae + *Spathelia excelsa*, subfamily Spathelioideae; BS 100%); (3) a clade containing members of the subfamily Aurantioideae (BS 100%) (*Severinia* +

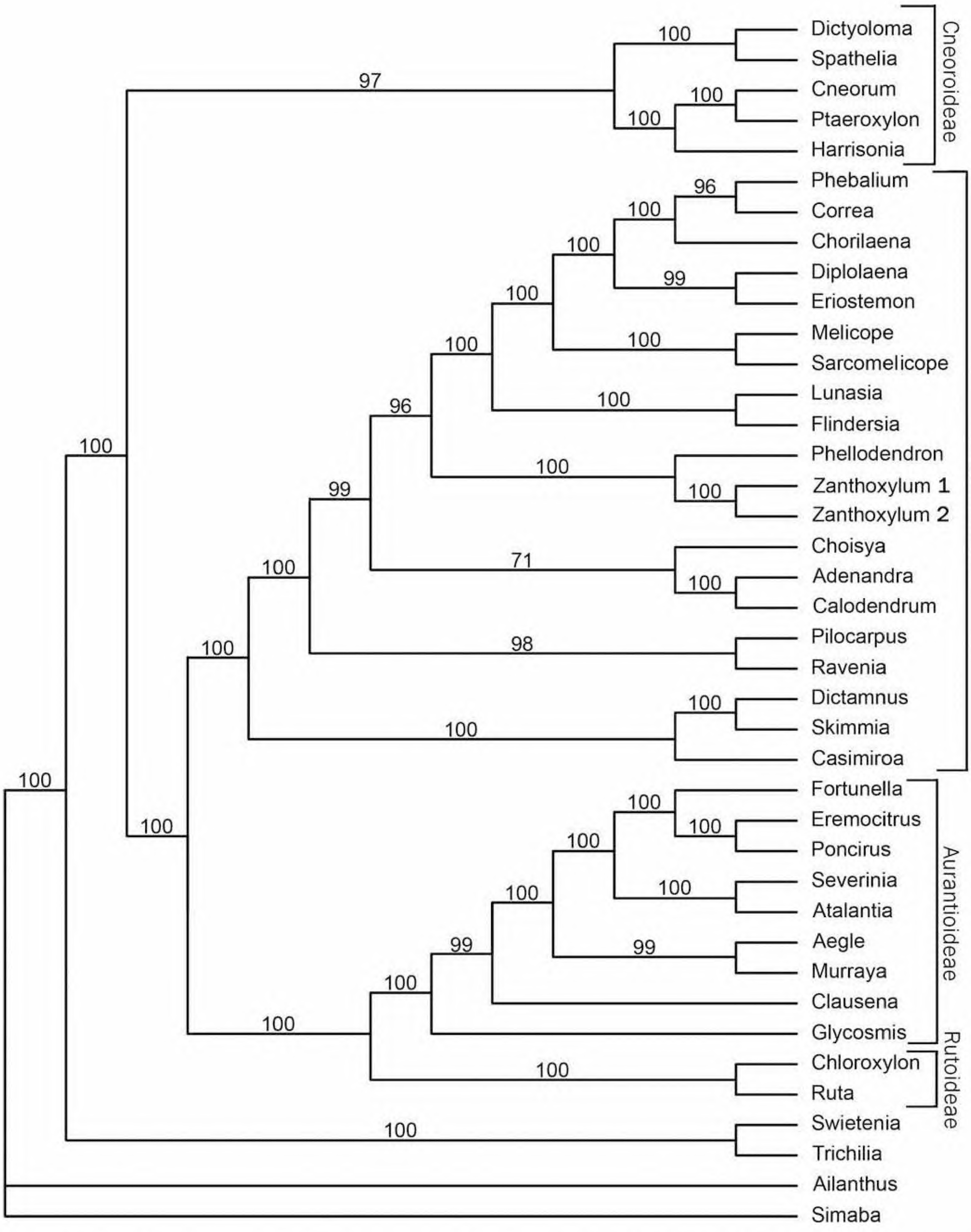

Figure 8. The Bayesian consensus tree from the combined of cpDNA and nrDNA analysis. Brackets indicate the taxa belonging to the four subfamilies. Numbers above nodes are posterior probability values.

Atalantia) + (*Fortunella* + (*Eremocitrus* + *Poncirus*; BS 77%) BS 87%). Basal to the subfamily Aurantioideae was the clade (*Chloroxylon swietenia* + *Ruta graveolens*; BS 100%); (4) a clade comprised of ((*Dictamnus albus* + *Skimmia anquetilia*; BS 91%) + *Casimiroa edulis*; BS 97%); (5) a clade containing (*Adenandra uniflora* + *Calodendrum capense*; BS 93%); (6) a clade containing two species of *Zanthoxylum* (BS 100%); (7) a clade containing (*Pilocarpus pennatifolius* + *Ravenia infelix*; BS 84%); (8) a clade consisting of (*Lunasia amara* + *Flindersia australis*; BS 99%); and (9) a well-supported clade (BS 94%) containing ((*Melicope* + *Sarcomelicope*; BS 100%) + ((*Phebalium*, *Correa*) + *Chorilaena*; BS 93%) + (*Diplolaena* + *Eriostemon*; BS 78%)).

Table 3. Summary of the results from separate and combined analyses of the *rbcL*, *atpB*, *atpB–bcL*, *trnL–trnF*, *rps16*, and *Xdh* region of Rutaceae.

Results	*rbcL*	*atpB*	*atpB–rbcL*	*trnL–trnF*	*rps16*	*Xdh*	Total
Total no. characters	1410	1489	987	1039	1054	1274	7240
No. variable characters (uninformative)	307	292	452	612	777	747	3259
No. parsimony-informative characters	157	153	222	427	479	462	1872
No. trees	18,352	9763	73,552	1654	2172	936	126
Tree length (steps)	406	353	657	1380	1643	1863	6295
Consistency index (CI)	50	53	53	56	59	59	48
Retention index (RI)	68	72	65	59	52	58	54
Clades with < 75% bootstrap values	7	9	6	12	5	15	22

In the Bayesian combined analysis, the group of exemplars from the Rutaceae were monophyletic with good posterior probability values (100%; Fig. 8). In all but one branch, the posterior probability values were higher than 96%. The only difference between the majority rule tree and the Bayesian tree was the sister group of *Adenandra uniflora* + *Calodendrum capense*. In the Bayesian analysis, *Choisya mollis* Standl. grouped with *Adenandra uniflora* + *Calodendrum capense* (posterior probability value 71%), whereas in the majority rule tree, *Adenandra uniflora* + *Calodendrum capense* formed a sister group with *Phellodendron amurense* and *Zanthoxylum* with very low support.

DISCUSSION

Classification of the Rutaceae has suffered from incomplete studies. To date, morphological data have provided very few clues to the true relationships of this large group of plants. Molecular data have provided some information; however, more evidence was needed to have a well-supported phylogeny. During the review of this paper, another treatment based on morphological features was published (Kubitzki et al., 2011). The Kubitzki et al. (2011) treatment recognizes three subfamilies, Cneoroideae, Rutoideae, and Aurantioideae, and differs from this study. In the current comprehensive study of the Rutaceae, molecular data and selected morphological characters were used to reclassify the subfamilies of this economically important group of plants.

SEQUENCE DIVERSIFICATION

The *rps16* sequences produced the most parsimony-informative characters for similar taxon sampling when compared with the other chloroplast or nuclear regions: *rbcL* (157), *atpB* (153), *atpB–rbcL* (222), *trnL-–trnF* (427), *rps16* (479), and *Xdh* (462) (see Table 3). The *atpB* gene produced the fewest parsimony-informative characters. The *Xdh* gene had the highest number of resolved nodes at or above 75% bootstrap support when compared with all other genes from the chloroplast regions: *rbcL* (7), *atpB* (9), *atpB–rbcL* (6), *trnL–trnF* (12), *rps16* (5), and *Xdh* (15) (see Table 3). Although the *rsp16* gene had the highest number of informative characters, it had the fewest nodes at or above 75% bootstrap support, indicating that the informative characters were clustering at only a few nodes. The *Xdh*, with almost the same number of informative characters, had the highest number of nodes at or above 75% bootstrap support. The combined parsimony analysis had 22 nodes at or above 75% bootstrap support, whereas in the Bayesian analyses all but one branch had posterior probability values higher than 96%. Because of the large number of nucleotide characters from the chloroplast and nuclear regions included in this study as well as the high levels of resolution and support from both parsimony and Bayesian analyses, the results were sufficiently robust to justify a reclassification at the subfamily level.

SUBFAMILY CLASSIFICATION FOR THE RUTACEAE

The subfamily classification proposed here requires major rearrangements: (1) the taxa of the subfamily Aurantioideae remain the same; (2) subfamily Cneoroideae encompass the subfamilies Spathelioideae, Dictyolomatoideae, *Harrisonia* of the Simaroubaceae, *Cneorum* of the Cneoraceae and *Ptaeroxylon* of the family Ptaeroxylaceae; (3) subfamily Rutoideae include *Ruta* and *Chloroxylon* of subfamily Flindersioideae; and (4) subfamily Amyridoideae Link unite *Flindersia* of subfamily Flindersioideae with subfamily Toddalioideae and taxa previously included in subfamily Rutoideae.

TAXONOMIC TREATMENT

Rutaceae Juss.

I. Rutaceae subfam. **Aurantioideae** Horan., Char. Ess. Reg. Veg., 203. 1847, as "Aurantiaceae." Aurantiaceae Juss., Gen. Pl. 259. 1789. Aurantiales Link, Handbuch 2: 345. 1829. Aurantieae Rchb., Fl. Germ. Excurs. 2(2): 840. 1832. Aurantiineae Rchb., Deut. Bot. Herb.-Buch, 88. 1841. TYPE: *Aurantium* Tourn. ex Mill.

Included genera (9). *Aegle* Corrêa, *Atalantia* Corrêa, *Clausena* Burm. f., *Eremocitrus* Swingle, *Fortunella* Swingle, *Glycosmis* Corrêa, *Murraya* L. Jack, *Poncirus* Raf., and *Severinia* Ten ex Endl.

The subfamily Aurantioideae comprise a strongly supported monophyletic group. This confirmed what has been found in previous phylogenetic studies (Chase et al., 1999; Scott et al., 2000; Samuel et al., 2001; Morton et al., 2003; Poon et al., 2007; Gruppo et al., 2008; Bayer et al., 2009; Morton, 2009). Morton (2009) examined 20 non-molecular characters, including vegetative, floral, biochemical, and chromosomal features and found only one character, the basic chromosome number of $x = 9$, provided a synapomorphy for the subfamily. Many of the morphological characters (e.g., flowers white, fruit with pulp vesicles) that had been used to define the subfamily previouly proved to be homoplasious. Morton (2009) confirmed the need for new morphological characters providing synapormorphies for classification at the subfamilial level. Other characters more recently suggested as potential subfamilial characters, such as the presence of coumarins, were homoplasious. Many of the characters traditionally used to describe subfamily Aurantioideae, such as seeds without endosperm, could not be used in a cladistic analysis because of the lack of information and plant material.

Bayer et al. (2009) and Morton (2009) found that the two tribes Clausenesae Wight & Arn. and Citreae Meisn. were not monophyletic but could be if *Murraya* and *Merrillia* Swingle were moved to the Citreae. Bayer et al. (2009) also found that none of the subtribes of Citreae are monophyletic, and before further taxonomic subtribal classification could be established, more molecular work is needed for a well-supported topology and classification. Relationships among species and genera, or groups, are complicated by several factors such as a long history of cultivation and wide cross-compatibility among species (Mabberley, 2004).

II. Rutaceae subfam. **Cneoroideae** Webb, London J. Bot. 1: 257. 1842. Cneoraceae Vest, Anleit. Stud. Bot. 267, 285. 1818. Cneorales Link, Handbuch 2: 440. 1829. Cneoreae Baill., Hist. Pl. 4: 431, 503. 1873. TYPE: *Cneorum* L.

Included genera (5). *Cneorum* L., *Dictyoloma* A. Juss., *Harrisonia* R. Br. ex A. Juss., *Ptaeroxylon* Eckl. & Zeyh., *Spathelia* L.

Within the parsimony analysis, both the pairing of *Dictyoloma* and *Spathelia* and the clade that included *Cneorum*, *Ptaeroxylon*, and *Harrisonia* (formerly Simaroubaceae) was strongly supported; however, the support of the sister relationship between these clades was weak. Within the Bayesian analyses, all of these clades had over 96% posterior probabilities. A synapomorphy for subfamily Cneoroideae is the presence of compounds known as rutacean chromones. Species of *Harrisonia*, *Spathelia*, and *Cneorum* all produce limonoids and ptaeroxylin chromones (Taylor, 1982, 1983; Da Silva & Gottlieb, 1987; Vieira et al., 1988, 1990), whereas *Ptaeroxylon* produces these chromones but no limonoids (Waterman, 1983). This relationship was also strongly supported in the studies by Chase et al. (1999) and Gruppo et al. (2008). Of the three applicable subfamily names, the subfamily Cneoroideae Webb (including *Cedrelopsis* Baill., *Harrisonia*, and *Ptaeroxylon*) was established in 1842, whereas Dictylomatoidae Engl. and Spatheloiodeae Engl. were described in 1896; therefore, the name Cneoroideae has priority over the other two names at subfamily rank.

III. Rutaceae subfam. **Rutoideae** Arn., Botany 104. 1832. Rutaceae Juss., Gen. Pl. 296. 1789. Rutales Juss. ex Bercht. & J. Presl, Přir. Rostlin 220. 1820. Ruteae Juss. in Dumortier, Anal. Fam. Pl. 45. 1829. TYPE: *Ruta* L.

Included genera (2). *Chloroxylon* DC., *Ruta* L.

Only two genera belong to the subfamily Rutoideae; the relationship of *Ruta* and *Chloroxylon* was strongly supported in both the parsimony and the Bayesian analyses. These genera have a strong synapomorphy of a base chromosome number of $x = 10$ (Stace et al., 1993). This base number has only been found in *Ruta*, *Chloroxylon*, and *Boenninghausenia*. Preliminary molecular data indicate that these three genera form a well-supported group (Morton, unpub.). This *Ruta* and *Chloroxylon* grouping has been found in several other studies that used different vouchered samples, representing different populations from different localities (Chase et al., 1999; Morton et al., 2003; Poon et al., 2007; Gruppo et al., 2008; Bayer et al., 2009; Morton, 2009).

IV. Rutaceae subfam. **Amyridoideae** Link, Handbuch 2: 128. Jan.–Aug. 1831. Amyridaceae Kunth, Ann. Sci. Nat. (Paris), 2: 353. 1824. Amyrideae DC., Prodr. 2: 81. 1825. Amyridales J. Presl, Nowočeská Bibl. 7: 335. 1846. Amyridineae Engl., Engler & Prantl, Nat.

Pflanzenfam. Teil III, Abt. 4–5: 111, 181. 1896. TYPE: *Amyris* P. Browne.

Included genera (19). *Adenandra* Willd., *Calodendrum* Thunb., *Casimiroa* La Llave, *Choisya* Kunth, *Chorilaena* Endl., *Correa* Andrews, *Dictamnus* L., *Diplolaena* R. Br., *Eriostemon* Sm., *Flindersia* R. Br., *Lunasia* Blanco, *Melicope* J. R. Forst. & G. Forst., *Phebalium* Vent., *Phellodendron* Rupr., *Pilocarpus* Vahl, *Ravenia* Vell., *Sarcomelicope* Engl., *Skimmia* Thunb., *Zanthoxylum* L.

The subfamily Amyridoideae consist of all the genera of subfamily Rutoideae (except *Ruta*), *Flindersia* (type for subfamily Flindersioideae, Handb. Syst. Bot. 2: 681. 1881, as "Flindersieae"), and the genera of subfamily Toddalioideae. These results support the conclusions of Gadek et al. (1996), Chase et al. (1999), Poon et al. (2007), and Gruppo et al. (2008), who stated that the subfamilies Toddalioideae and Rutoideae must be unified into one subfamily. The oldest subfamily name that excludes the genus *Ruta* is subfamily Amyridoideae published in 1831 and, therefore, by priority is the name to use for this group of genera. Traditionally, Engler (1931) separated subfamily Toddalioideae from subfamily Rutoideae by fruit characters. However, revisionary studies by Hartley (1974, 1981, 1982) indicated that genera of subfamily Toddalioideae with syncarpous, drupaceous fruits are more closely related to genera of subfamily Rutoideae with apocarpous or subapocarpous follicular fruits. A study of ovule and seed-coat patterns found little distinction between the two subfamilies (Boesewinkel, 1980). While biochemical studies by Fish and Waterman (1973) and later by Da Silva et al. (1988) provided some evidence of a link between the subfamilies Rutoideae and Toddalioideae, to date there are no morphological, anatomical, or biochemical synapomorphies for the union of the two subfamilies. Additional molecular and morphological work is needed to resolve relationships among subfamily Amyridoideae. Gruppo et al. (2008) suggested that this clade appeared to be correlated with the geographic distribution of the groups, although this pattern was not clear in this analysis. The Old World taxa appear to be interdigitated with taxa of the New World. The grouping of *Dictamnus* with *Casimiroa* and *Skimmia* as a clade was also found by Chase et al. (1999) and by Gruppo et al. (2008), although no morphological or biochemical characters supported this grouping. The clade consisting of *Zanthoxylum* and *Phellodendron* was also found in a study by Chase et al. (1999, *atpB* clade) and by Poon et al. (2007), with support by one character of 1-BT1Q alkaloids (Waterman, 1975).

Conclusion and Future Plans

Four subfamilies, all monophyletic, are now recognized within the Rutaceae: (1) subfamily Aurantioideae, (2) subfamily Cneoroideae, (3) subfamily Rutoideae, and (4) subfamily Amyridoideae. Within these four subfamilies, resolution and good bootstrap support and posterior probability values are recovered for many of the clades. The most significant phylogenetic ambiguities remain in the subfamily Amyridoideae, from which only 19 of the approximately 120 genera have been sampled. To address these problems and to further consolidate results, the author plans to expand the taxon sample to include more genera within subfamily Amyridoideae. The author is confident that the relationships presented here will be further supported by the addition of new data and that these revised subfamily circumscriptions represent significant steps toward a natural and stable classification that will stand the test of time and aid additional studies of these diverse and important taxa.

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